Targeted Nanomedicines for Cancer Therapy, From Basics to Clinical Trials

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ABSTARCT - Traditional systemic chemotherapy involves the wide distribution of drug molecules in the body, causing toxic side effects in the healthy tissues and limiting the therapeutic dose required at the site of drug action. In order to decrease side effects and increase the drug efficacy, recent research on chemotherapy focuses on drug targeting. Targeted therapy can be achieved by several mechanisms including; 1) using an antibody as a drug that is specific to a disease biomarker, 2) using an antibody (or peptide) as a targeting agent conjugated to the drug molecule, 3) delivering the drug molecules to the target tissue in a nano-carrier with or without the targeting agent attached on its surface. The third approach involves the nanomedicines that can be targeted to diseased tissues by both passive (extravasating at diseased sites due to leaky vasculature) and active (specific interaction of the targeting agent with disease biomarker) targeting mechanisms. In this review we will cover the passively targeted nanomedicines prepared using nano drug carriers. Ideally the carrier particle should be in the right size (1-100nm), stable enough to prevent drug leakage during circulation, and safe not to cause any damage to healthy tissues. Competition for all these properties generated many different types of materials to be used as nanodrug delivery systems. After a brief review of most commonly used drug carriers, we discuss the clinical use of the targeted nanomedicines with regard to their pharmacokinetic and pharmacodynamics properties, and how these properties vary from conventional formulations providing free drugs in the circulation after administration.

INTRODUCTION

In systemic drug therapy, the drug is distributed throughout the body via the bloodstream and only a small amount of the administered drug can reach the diseased tissue. Depending on the drug nature, drug molecules in the body may go to different regions in the body, dissociate in healthy tissues, interact with the neighbouring cells or be metabolized and excreted from the body. It is very common for the drug molecules that cannot reach their target to form toxic side effects. Treatment dose of the drug given to the body is adjusted according to these toxic effects. However, the expected pharmacological effect of the drug is dependent on the drug concentration in the diseased area and the dose required for complete treatment is not easily administered in some cases. For example, in the treatment of cancer, the drug is re-administered after the expected side effects are ameliorated. The administration of the drug in small and repeated doses constitutes immunity against drug in the cancer cells and causes more rapid proliferation of cancer cells compared to normal cells. In order to find a solution for this serious drug resistance problem, targeted treatment methods have been developed in recent years (1).

The aim of targeted therapy is to ensure that the drug molecules are concentrated predominantly in the affected area and distributed as little as possible to other parts of the body.

Drug targeting can be achieved in a variety of ways, such as using a molecule (or antibody) as a drug that directly interacts with a particular biomarker in one disease without interference with other tissues, linking the drug molecule to an antibody that directly interacts with a disease-specific biomarker or loading drug molecules into a nano-carrier and sending them to the diseased tissue via passive and active mechanisms. In targeted therapy the effect of the drug increases as significant amounts of the dosage given is collected in the diseased area and unwanted side effects on other tissues are therefore reduced. With this approach, the possibility of using a higher drug dose and more effective treatment can be achieved. In addition, the patient's quality of life is greatly increased due to reduced side effects in targeted treatment.

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This review briefly describes the different types of nanocarriers, their targeting methods to the desired regions in the body and how pharmacokinetic and pharmacodynamic properties of targeted nanodrugs differ from conventional drugs.

NANOPARTICLES

The term "nano" is derived from the Greek word "nanos", which means dwarf. One nanometer is equal to 10^{-9} m and the nanoparticles used in medicine are expected to have dimensions of 1-100 nm. However, due to the difficulty of preparing particles at this size and the use of larger nanoparticles in the industry, according to the new scopes, materials with at least one dimension larger than 1 nanometer and smaller than 1 micron can be referred to as nanoparticles (2, 3). The nanoscale production of materials has positively influenced industrial fields such as agriculture, food and textile. Specifically, extraordinary progress has been recorded in medical applications of nanoparticles. Nanoscale materials can have a range of medical applications in drug delivery systems, imaging, implants and diagnoses (4). In addition, due to their functional and visual advantages including solubility improvement and possibility for topical application, nanoparticles' usage in cosmetic sector is becoming increasingly widespread (5).

Using nanotechnology and nano-carriers for the purpose of drug delivery has the following advantages:

A: Development of drug characteristics:
- Increasing solubility and stability (6)
- Enhancing biodistribution and bioavailability as a result of targeted drug delivery (7)

B: Development of dose-dependent features:
- Decreasing applied dose
- Decreasing or even eliminating unwanted side effects

So far, various nanomaterials with the above-mentioned characteristics have been developed to deliver drugs and currently new carriers are under investigation. Two different approaches are generally adopted in the preparation of nanoparticles: top-down and bottom-up.

In top-down method, large materials being of micron size are converted to nano size using different methods like milling, homogenization and sonication. However, in bottom-up method, the aim is to ensure that the substances at the molecular level rise to nanometer size through various methods such as chemical synthesis and self-assembly in solutions (3, 8).

Nanosized drug delivery systems and their properties that are frequently used and explored in health sciences are investigated in the following part. Fig. 1 presents the shapes of some of the nano drug delivery systems described in this review. Table 1 summarizes the uses and content of these nano drug delivery systems.

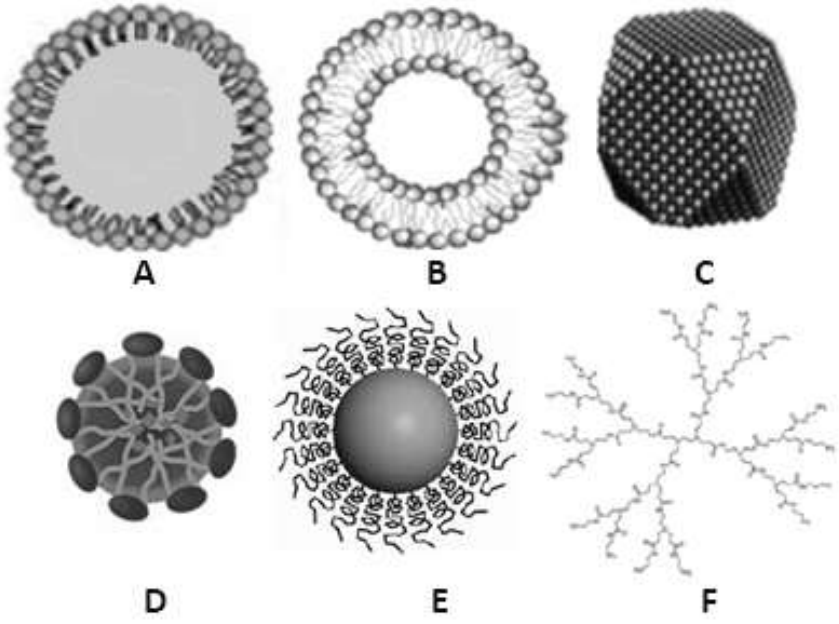

Fig. 1: The shape of some nanoparticles described in this review; A) Lipid Nanoparticle, B) Liposome, C) Nanocrystal, D) Micelle, E) Gold Nanoparticle and F) Dendrimer (9)

Table 1. Contents of the nanoparticles described in this review and their medical uses

Nanoparticle	Material	Medical Uses
Liposomes, Niosomes	Phospholipids, Non-ionic Surfactant	Drug / Gene delivery, Cosmetics
Micelles	Polymer, Phospholipid Surfactant	Drug / Gene delivery, Surface Cleaning, Cosmetics
Nanoemulsions, Microemulsions	Lipid + Surfactant, Lipid + Surfactant + Co-surfactant	Drug / Gene delivery, Cosmetics
Solid Lipid Nanoparticles, Nanostructured Lipid Carriers	Solid Lipid + Stabilizer, Solid Lipid + Liquid, Lipid + Stabilizer	Drug / Gene delivery, Cosmetics
Dendrimers	Branched Polymer	Drug / Gene delivery,
Polymeric Nanoparticles	Polymer	Drug / Gene delivery,
Nanocrystals	Drug	High Dose Drug Delivery
Quantum Dots	Cdse, Zns, Inas, Pbs, Gan	Imaging
Carbon-Based Nanoparticles	Carbon	Drug / Gene delivery,
Metallic Nanoparticles	Gold, Silver, Iron Oxide And Similar Metals	Imaging, Drug / Gene delivery, Photothermal or Magnetic Therapy

Liposomes

Liposomes are spherical vesicular systems with an inner aqueous core and lipid bilayer structure with a size of 0.02-3.5 μm. They are usually prepared with amphiphilic phospholipid molecules. Addition of various sterols, especially cholesterol, to the bilayer structure increases the stabilization of liposomes and drug delivery capacity. Liposomes show structural similarity to phospholipids or lipoproteins found naturally in cell membranes. Due to these properties, liposomes are biologically compatible and can be degraded in the body and do not lead to serious toxic effects. Oxidation and hydrolysis of lipids during storage time and the tendency of the particles to grow together over time are among the common stability problems of liposomes (10, 11).

With the aim of avoiding these stability problems, nisosomes have been emerged as an alternative drug delivery system that can also provide an economical alternative to the high cost of phospholipids. Niosomes are vesicular systems, prepared with double layers of non-ionic surface active materials with methods similar to liposomes. The toxic effects of surfactants in the structure and the risk of fusion and aggregation during storage are among the disadvantages of nisosom-based systems (12).

Liposomes are used to enhance the solubility, bioavailability, systemic retention, or tissue-related distribution of encapsulated drugs (13-15). Liposome-based treatments are known to broad the therapeutic window significantly. Especially in oncology, these drug delivery systems are very effective due to their selective targeting to the solid tumors (16-19).

For example, Hempel et al. investigated pharmacokinetics of daunorubicin and Daunoxome® (liposomal daunorubicin) in children. Data was reported to best be described by a one compartment model. Clearance was found to be 6.41 ml h^{-1} kg^{-1}± 0.5 51% and volume of distribution 65.4 ml kg^{-1} ± 0.5. The area under the curve at a dose of 60 mg m^{-2} was 231 mg l^{-1}h. Overall, DaunoXome® showed prolonged retention, increased target distribution, equivalent efficacy, and reduced toxicity in comparison with Daunorubicin (20).

Cytarabine is the active ingredient of DepoCyt. In treatment with DepoCyt®, tumour exposure to the cytotoxic concentration and response rate increased while toxicity decreased. Similarly, a decrease in toxicity was observed in Doxil® and Myocet®, retention and target distribution were increased and an equal efficacy with Doxorubicin was achieved. In Marqibo®, even a superior efficacy in comparison with the active substance, Vincristine, was observed. These kinds of advantages are mentioned for some other approved liposome formulations like Vyxeos®, Onivyde™ and Mepact®.

Batist compiled cardiac safety of liposomal anthracyclines (21). Despite very striking clinical efficacy of this chemotherapy agents, synergistic cardiac toxicity limits the application. Liposomal encapsulation is a strategy to deliver these drugs

while keeping heart safe from the side effects. Stability, drug release rate, and in pharmacokinetic properties, such as blood circulation time, sites of deposition of the liposome are mentioned as advantages (22).

In a series of clinical trials, Doxil/Caelyx has been shown to have significant efficacy in breast cancer treatment with reduced cardiac toxicity (23).

Myocet (non-pegylated liposomal doxorubicin) was compared to equivalence dose of free doxorubicin in breast cancer in two randomized trials (24, 25). The formulation enhanced the anti-tumour efficacy and lowered the cardiac toxicity. Table 3 gives more examples of liposomal formulations.

Micelles

Micelles are small size (5-100nm) particles formed by spontaneous assembly of polymeric or lipid-based amphiphilic molecules in the aqueous media, without requiring any external energy. The interior part of spheriods is hydropobic and serves as a carrier for oil soluble drugs and the outer part is hydrophilic (26, 27). Amphiphilic molecules form micelles in the aqueous medium only after reaching a certain concentration and aggregation may happen in this process so that the free energy of the system is reduced. This threshold concentration value is called the critical micelle concentration (CMC) and differs depending on the structure of amphiphilic molecules. Systems with a low CMC are more resistant to dilution. When an aqueous dispersing system containing a micelle is diluted with water, the concentration of amphiphilic molecules present as monomer in the system is reduced and the micelle is broken down to the amount required to restore the concentration to the CMC value. In this case, the drug loaded into the micelles also releases in the aqueous medium. After injection and contact with large blood volume, micelles with high CMC may cause precipitation with sudden release of the drug, which may result in embolism. Because of these reasons, low CMC values are preferred in treatments.

Micelles are preferred drug carriers because they are easy to prepare, able to carry high amounts of drug, highly stable in their structure and modifiable to serve different purposes (27). In addition, micelles prepared by phospholipids demonstrate an acceptable toxicity profile (28).

Genexol-PM and Nanoxel-PM are micellar formulations that were approved in South Korea. In their study, Kim et al. (29) compared Genexol-PM with conventional paclitaxel. They found Genexol-PM advantageous to paclitaxel due to lack of necessity for pre-medication and ability to deliver more drugs with no extra toxicity.

Lee et al. (30) developed and evaluated Nanoxel-PM in comparison with its conventional form, Taxotere®. In studies in rats, mice and beagle dogs, Nanoxel-PM showed similar pharmacokinetic profiles to Taxotere® and unmetabolized docetaxel was found to be the same in both cases in excreted drug in faeces or urine. Comparable efficacy was achieved with pharmacokinetic bioequivalences in lung cancer *in vivo* and lung, ovary and breast cancer cell lines. Also, similar toxic effects are reported in related studies. Table 4 shows more information on similar micelle formulations.

Nanoemulsions

Nanoemulsions are drug delivery systems, usually 10-200 nm in size, prepared by homogeneous distribution of two incompatible liquid phases. A variety of amphiphilic surfactants are added to the formulation and mechanical shear is used to provide emulsion formation. Based on the desired application, nanoemulsions can be prepared as oil in water (O/W) or water in oil (W/O) (31, 32).

The nanosize of the emulsions that have oil droplets in their internal phase allows the parenteral application of oil soluble drugs and various vitamins. The most common stability problem in O/W nanoemulsions is the Ostwald repening and the dissolving of small sized particles of the system in the outer phase and their participation in the formation of larger particles over time.

Flocculation is more commonly seen in W/O emulsions and is known as the association of small particles with large particles and the increase in particle size.

The relatively low amount of surfactant required for the preparation of emulsions, their applicability in the form of cream, spray, foam and lotion and their resilience against dilution and pH changes are among the advantages of these systems (32).

Microemulsions are also colloidal dispersions with a droplet size of 5-200 nm. They are formed spontaneously by mixing high amounts of surfactant and co-surfactant without any need to external energy. Triangular phase diagrams are often used to determine the necessary amounts of surfactants during the preparation process. Microemulsions are thermodynamically stable, but the high level of surfactant present in the structure can lead to various side effects (33).

An example of this type of nanoformulation is Oncaspar®. Oncaspar® was approved by EMA in 2016 to treat Acute lymphocytic leukemia (ALL). Panetta et al. (34) checked the pharmacokinetics and pharmacodynamics of Asparaginase and PEG Asparaginase (Oncaspar®) in ALL. The clearance of native asparaginase was much higher than that of PEG asparaginase. They found significant pharmacokinetic and pharmacodynamic differences due to asparaginase preparation for example VMAX decreased from 122 to 61 (μM/days)/(IU/mL), KmCSF from 3.6 to 1.1 μM and kin (1/days) (4.8 to 1.0) by PEGylation.

A nanoemulsion of 5-Aminolevulinic acid is in clinical trial for superficial basal cancer cell photodynamic therapy (35). Three photosensitizers are compared in phase 2. The photodynamic is joined with aminolevulinic acid nanoemulsion (BF-200 ALA/Ameluz®) and two other compounds.

The nanoemulsion is 7.8% of 5-aminolevulinic acid and in order to rise the affinity towards epidermal tissues soy phosphatidylcholine and propylene glycol are used (36). The clinical trial status is Active. The same formulation was used for treatment of lentigo maligna (NCT02685592) and some other skin disorders like multiple actinic keratosis (NCT01893203) and actinic keratosis (NCT01966120 and NCT02799069).

Lipid nanoparticles

Lipid nanoparticles can be of two types, namely solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC). SLNs are disperse systems of 50-1000 nm size, prepared by stabilizing various solid lipids using different surfactants in an aqueous environment. NLCs are a new generation of lipid nanoparticles, which contain liquid lipid in core surrounded by solid lipid. Fatty triglycerides, fatty acids, sterols and various waxes are used as solid fat, while oleic acid and vegetable triglycerides with medium chain length are generally preferred as the liquid lipid.

Depending on the production method and the nature of the lipids used, the active substance may be dispersed in the matrix or it may be present in the core or shell part. Release from particles also varies according to the medium in which the drug is present. When the drug molecules accumulate on the surface, a rapid release is observed while the drug molecules trapped in the inner part exhibit a slower and controlled release. SLNs generally have less drug loading capacity than NLCs due to the lipid phase density. Leakage of the drug from carrier over time and different colloidal constructions that may be present in the environment after production are among the disadvantages of these systems. Nonetheless, lipid nanoparticles are preferred because of their low toxicity, the absence of organic solvents during their preparation and their stability for large scale production and sterilization (37, 38).

Liu et al. (39) reported successful avoid of resistance to paclitaxel in breast cancer cells by delivering miRNA-200c in cationic SLNs. SLNs transfected the MCF-7 cells more effectively than Lipofectamine or the free miRNA-200c without changing morphology and environment of the cells. They concluded that this treatment can improve IC50 of paclitaxel and delivery of miRNA in breast cancer treatment.

Ji et al. (40) used SLNs to deliver naringenin. Although naringenin didn't affect the viability of A549 cells, naringenin-SLN presented high cellular uptake. In biodistribution studies in rats, intratracheal instillation administration of naringenin-SLN improved pharmacokinetic parameters like mean residence time and maximum plasma concentration.

In another study, folic acid functionalized SLNs was used for oxaliplatin delivery for treatment of colon cancer. Functionalized SLNs showed the best anticancer potential in comparison to nonfunctionalized SLNs and free drug (41).

Dendrimers

Dendrimers are spherical synthetic constructs consisting of functional groups branching around the nucleus. Dendrimer name is derived from the word "dendro", which means "tree" in Greek. Dendrimers are classified by different generation numbers which refer to the number of repeated branching cycles that are performed during their synthesis. As the number of branching points increases, the molecule size increases as well.

Drug delivery in dendrimers occurs by the encapsulation of the drug in the denderimeric structure or by the interaction with the functional groups on the surface. Through modification of these groups, hydrophobic or hydrophilic types of the molecule can be prepared and various ligands can be added for targeted drug delivery. The molecule size and weight of the dendrimers can be controlled as desired during product synthesis and it is possible to design them for different applications. However, multiple stages in the production of bigger molecules increase costs and make the industrial production difficult on larger scales (42). In addition, toxicity profiles of dendrimers in the body must be taken into consideration.

Dendrimers are known to be able to enhance the uptake by cells, bioavailability and therapeutic efficacy. They also can optimize the biodistribution and intracellular release profile. Clearance and degradation rate of attached drugs and toxicity are reported to be reduced (43). Although there is not any approved dendrimer formulation by FDA or EMA, there are some formulations in clinical trials. For example, DTXSPL8783 is a docetaxel (DTX)-dendrimer conjugate in phase 1 study in patients with advanced solid tumours in UK (44)

Several dendrimeric formulations are reported to be successful *in vitro* and *vivo* experiments. For example, Al-Jamal KT (45) reported the use of a cationic poly-L-lysine dendrimer complexes of doxorubicin compared to the free DOX. The complex is reported to show better penetrability into monolayers than the multicellular tumour spheroids, and *in vivo* tumors than the free drug. Toxicity was reduced significantly in complexation use.

Han et al. (46) used a combination of chemotherapy and gene therapy for the treatment of liver cancer. A T7-conjugated polyethylene glycol-modified polyamidoamine dendrimer (PAMAM-PEG-T7) was used as a carrier to co deliver doxorubicin and the therapeutic gene encoding human tumor necrosis factor-related apoptosis-inducing ligand (pORF-hTRAIL). In Bel-7402 cells, T7-modified system had higher uptake and gene expression than unmodified system. Higher accumulation and efficiency was observed *in vivo* as well in comparison with free DOX or pORF-hTRAIL.

In another study, PAMAM-based dendrimers were used by Lee et al. (47) as vehicles to combine chemotherapy and immunotherapy for prostate cancer. They reported that the system with CpG oligonucleotides (ONTs) as immune-stimulants and doxorubicin as chemotherapeutic agent showed much lower toxicity than the same dose of free Dox in *in vivo* murine tumour models.

Polymeric nanoparticles

Polymeric nanoparticles, as the name implies, are drug delivery systems prepared with different polymers. The polymers used can be classified as biodegradable or non-biodegradable. The first class contains natural polymers (e.g., gelatin, chitosan) and the second class includes synthetic polymers (e.g., polylactic acid and PLA). Where non-biodegradable polymers are used, those having low toxicity such as polyethyleneglycol (PEG), polyvinylpyrrolidone (PVP) and carboxyl methyl cellulose (CMC) are generally preferred for medical applications (48).

Polymeric nanoparticles can be prepared in different forms such as nanospheres and nanocapsules. Commonly used nanospheres are spherical drug delivery systems composed of a polymeric matrix. Drug delivery occurs by erosion and/or diffusion depending on the nature of the polymer. Nanocapsules form a polymeric outer shell over a lipophilic inner core. Drug release rate is slower than nanospheres due to the polymeric membrane but the drug carrying capacity is higher in the core.

Drug molecules may be carried inside polymeric matrix or adsorbed to the NP surface. These systems may also be prepared as polymer-drug or polymer-protein conjugates obtained by chemically linking the polymer and the active molecule. This binding can take place directly if there is sufficient interaction between the drug and the polymer, but it is usually done using a variety of binding molecules.

It is expected that at the targeted site, the molecules that bind polymer and drug are broken down and the drug is released, which leads to the desired effect. This degredation may occur enzymatically or via hydrolysis in the biological medium (49). The prepared NPs can also release the drug in the presence of various stimuli such as heat, pH or light, depending on the nature of the polymer used.

Advantages and disadvantages of polymeric drug delivery systems depend on physiochemical properties of different polymers used and the production techniques (50).

One of the best examples in this group is Abraxane. The hydrophobic drug paclitaxel is delivered by bounding to Albumin. Biodegradability, lack of toxicity and immunogenicity and good facilitated uptake in tumor and inflamed tissue are among the properties that make Albumin a good carrier (51). The bioavailability of paclitaxel is highly boosted in Abraxane that resulted in better intra-tumor concentrations assisted by albumin-receptor (gp60) mediated endothelial transcytosis (52-54). It undergoes biphasic elimination (two-compartment model of disposition) with a terminal half- life of 27 hours (5.8 hours for paclitaxel). The clearance is 43% slower (15 L/h/m2) and the mean volume of distribution is 632 L/m2 (indicating extensive extravascular distribution). The drug exposure (AUC) was proportional to the dose in the range of 80- 375 mg/m2. Reconstitution potential of Abraxabne in saline is increased to 2–10 mg/ml from that of 0.3–1.2 mg/ml in paclitaxel.

The terminal half- life of 5.8 hours in paclitaxel was also increased to 27 hours in Abraxabne. The clearance was 43% slower (15 L/h/m2) and extravascular distribution was increased (mean volume of distribution is 632 L/m2). The drug exposure (AUC) was proportional to the dose in the range of 80- 375 mg/m2 (55) . In 2005, FDA approved Abraxane for metastatic breast cancer in the first place. Later in 2012, it was approved for advanced non-small cell lung cancer and advanced pancreatic cancer. Abraxane was also approved by EMA for metastatic pancreatic cancer in 2013.

Genexol-PM that was discussed under micelles title is a good example of polymeric micelles. CRLX101 is a drug–conjugate formulation of camptothecin and a cyclodextran-PEG polymer that was studied in several clinical trials for different types of cancer (44)

Weiss et al. (56) reported the first-in-human phase 1/2a trial of CRLX101 in patients with advanced solid tumors. Active ingredient of CRLX101 is Camptothecin (CPT) that works via interaction with DNA. However, this interaction is non-covalent and reverses within minutes of drug removal. Furthermore, CPT is known to cause considerable toxicity, including diarrhoea and myelosuppression. In CRLX101, cyclodextrin-containing polymer (CDP) is conjugated to CPT. This conjugation increases the solubility by three orders and decreases the inactivation rate. Both in preclinical studies and human models, renal clearance was decreased but plasma half-life was increased in conjugated form of CPT (57-60). With CRLX101 CPT, accumulation in tumor site was increased (61) and prolonged release of CPT enhanced the antitumor activity. In both pharmacokinetic and excretion studies, polymer-conjugated form showed promising results in comparison with unconjugated form. Extended slowly release of drug from polymer lead to sustained Cmax. AUCall data showed that conjugation increased CPT exposure by about eleven fold. Majority of excreted drug was in conjugated form (16.2 % of the total CRLX101 vs 4.4 % unconjugated). Table 4 gives more examples of this group of nanoparticles.

Nanocrystal*s*

Drug nanocrystals are crystal structures with particle sizes in the nanometer region. Nanocrystals consist of 100% drug molecules and since they are in nano size, they do not need any careers. The surface area of nanocrystals is more than that of the micron-sized particles. This large surface area causes an increase in the dissolution rate of particles and hence an increase in bio-availability. Dispersion of nanocrystals in an aqueous medium is called nano-suspension. Generally, stabilization of the dispersed particles must be ensured, therefore various surfactants or different polymers are added to the dispersion medium.

As they can be used orally in tablets and capsules, nanocrystals may also be administered parenterally due to their small particle size (62). As a nanocrystal, the active ingredient given to the body needs to dissolve in the target tissue and turn into a molecular state in order to be effective. Also, consideration should be given to the toxicity of nanoparticles going to the regions other than the target tissue.

Although there are several FDA approved nanocrystals for different diseases, there is not any anticancer among them yet. However, some cases have gone through clinical trials (63).

Panzem® (Nanocrystalline 2-methoxyestradiol) is an example that was used for different types of cancer. Harrison et al. (64) conducted a trial to evaluate the medicine for the treatment of metastatic castrate-resistant prostate cancer. Despite promising pre-clinical results, in this study, the formulation didn't show significantly successful results. However, it is reported to show biological activity and to be well tolerated. It is concluded that this unpromising results can be due to highly aggressive nature and level of disease.

Thymectacin is another poorly soluble drug that was made bioavailable as nanocrystal under trade name of Theralux®. Eradication of cancerous cells from bone marrow transplants in non-Hodgkin's lymphoma was one of areas that the drug was used.

NBTXR3 is a crystalline solution of hafnium oxide nanoparticles. Tourneau et al. (65) reported diminish in tumor size when they used NBTXR3 upon exposure to radiotherapy compared to just radiotherapy.

Quantum dots

Quantum dots are semiconductor nanocrystals with 2-10 nm dimensions. Their optical and electrical properties are different from other nanoparticles because of their very small size. Small-sized particles (2-3 nm) radiate in short wavelength and appear blue-green. Larger particles (5-6 nm) radiate in longer wavelengths and appear orange-red. The number of electrons, structure and shape of particles can be changed in the desired format. Because of these properties, they are investigated in medicine mainly in imaging and early diagnosis. Various

investigations on toxicity of quantum dots have been conducted but due to the diversity and unique behaviour of the materials used in their production, no definite result has been achieved (66).

Olerile et al. (67) reported co-delivery of PTX CdTe@ CdS@ZnS QDs using lipid carriers. Encapsulation efficacy was reported to be ~80% while drug loading and tumor growth inhibition rate were 4.68% and 77.85%, respectively.

Zhao et al. (68) reported another platform for chemotherapy and fluorescence imaging. PTX was loaded to the hydrophobic inner core while coated with a hydrophilic silica shell and ZnSe:Mn@ZnS QDs. The targeting responsible part was amino groups on the surface. The system increased the solubility of PTX in a 630 order and it sustain released in 12 h.

Cai X et al. (69) targeted overexpressed glycoprotein CD44 in cancer cells with polyethylene glycol (PEG) and hyaluronic acid functionalized pH-responsive ZnO QDs loaded with Doxorubicin. The system was reported to release the drug under acidic intracellular conditions. Integration of anticancer effect of Zn^{2+} and DOX led to a synergistic therapy.

Fullerenes

Fullerenes are empty, spherical, elliptical or cylindrical shaped carbon-based particles. Fullerenes have different number of carbon atoms but the most well-known and first produced fullerene structure has 60 carbons. Spherical fullerenes, also called Buckyball, have a particle size of about 1 nm (70). Cylindrical fullerenes are called carbon nanotubes or "buckytubes" (71). Carbon nanotubes, which can be found as single-layer or multi-layer, are among the most durable materials known. Unmodified carbon nanoparticles are insoluble in water, so surface modification is required to achieve water solubility and reduce cytotoxicity. Carbon-based nano-carriers easily penetrate into cells due to their hydrophobic nature, but they have been shown to have negative effects on the immune system (72).

Zakharian et al. (73) designed a C60-PTX to target lung cancer. Bioavailability and therapeutic efficacy of PTX was improved. Half-life of PTX in bovine serum was 80 min and the PTX release took place by enzymatic hydrolysis.

Another successful C60 conjugation via a carbamate linker with doxorubicin was reported by Chaudhuri et al. (74). High *in vitro* and *in vivo* activity without systemic toxicity like free DOX is reported.

Metallic nanoparticles

Metallic nanoparticles are generally colloidal drug delivery systems prepared using soil elements such as gold, platinum, silver, copper and iron oxide. Metallic nanoparticles can be prepared in different sizes and shapes, and their surfaces can easily be modified and functionalized with different molecules. Metallic nanoparticles have properties that vary according to the element used.

Silver nanoparticles have effective antimicrobial and antioxidant properties (75), while gold nanoparticles are used for imaging, early detection of tumors and also in thermal ablation therapy due to their unique optical and photothermal properties (76). Iron-containing nanoparticles are used for their magnetic properties. Externally applied magnetic field after application can ensure that the iron nanoparticles accumulate in the desired region (77).

The biggest disadvantage of metallic nanoparticles is that they are not biodegradable and have the risk of accumulation in the body. Toxic effects vary depending on the element and particle size used (3).

Aurimmune is a coated gold nanoparticle with thiolated PEG that was coupled to TNF-α for targeted delivery to tumor. This was the first successful attempt without dose-limiting toxicity that led to hypotension and nausea. Even when dose levels as high as 500-600 microgram/m^2 of TNF-α was used, the side effect was limited to grade 2 fever (78).

Libutti et al. (79) also reported promising results of Phase I and pharmacokinetic studies of Aurimmune (CYT-6091) previously. PK analysis was performed for total rhTNF as it is not possible to assay for bound versus free rhTNF, nor is it possible to quantify the amount of gold based upon the sensitivity of available assay techniques.

AuroShell is another example that went through clinical trials. A thin layer of gold is coated on silica particles. Several studies reported successful use of AuroShell in photothermal therapy or along with radiation or standard chemotherapy (78, 80-84).

Gad et al. (85) evaluated the toxicity of AuroShell when intravenously delivered. Evaluation was based on internationally recognized tests *in vivo* and *in vitro*. Nanoshells were totally well tolerated and biocompatible and in none of the studied toxicity was observed.

PEGylation

One of the biggest problems faced with nanoparticles after they enter the blood circulation is their recognition as foreign materials and

elimination by opsonization. Complement protein is an opsonin type protein and is stimulated by the entry of foreign materials into the body. As a result of this warning, the complement protein interacts with the foreign particles surface and causes the particles to be eliminated by the macrophages. If the surface of the particles is coated with a hydrophilic polymer prior to the administration, this opsonization event can be reduced or even completely prevented. One of the most suitable polymers used to coat the particle's surface is the PEG polymer, and this coating process is referred to as PEGylation; (coating nanoparticle surface with poly ethylene glycol (PEG)).

PEG chains are attached to the surface of the particle by chemical bonding or by physical interactions. PEG is a hydrophilic polymer, which causes the accumulation of a dense water layer on the surface of the nanoparticle. The thickness of this layer and PEG coverage of the surface directly affect the opsonization level of the particles.

PEG polymers that cover the entire surface of the particles in a dense manner provide good protection against opsonins as they will form a thick water layer. However, a small number of PEG chains may not be effective enough (Fig. 2) (86, 87). Nanomedicines prepared with PEGylation stay for a longer time in the circulation and are more likely to reach the desired target.

Genexol-PM that was mentioned under micelle group and also Oncaspar® that was mentioned under nanoemulsion are PEGylated.

Sylatron™(PEG-INTRON®) is the PEGylated version of interferon-α2b that got FDA approval to be used for adjuvant therapy in treatment of melanoma (88). The half-life was found to be 27–37 h, clearance decreased 10 fold and a minor change was observed in comparison with non-PEGylated form.

Thermodox is another formulation that went through clinical trials for treatment of hepatobiliary tumors (89) . It is a thermosensitive, PEG bounded, liposomal doxorubicin that releases the drug when exposed to high heat (44).

CALLA 01 by Calando Pharmaceuticals is transferrin conjugated cyclodextrin nanoparticle coated with PEG. It was the first formulation to go under phase I clinical trials for solid tumors (90).

Kurmi et al. (91) delivered methotrexate to lung cancer using Lactoferrin-conjugated dendrimers and compared it with the free form. pharmacokinetic and pharmacodynamic properties were also investigated. Elimination half-life of MTX-loaded plain PEGylated poly (propylene imine) (PPI) dendrimer (10.41 ± 2.12 h, $p < 0.05$) increased (12.23 ± 1.53 h, $p < 0.01$) MTX-loaded Lf-conjugated PEGylated PPI dendrimer. Overall, a prolonged systemic exposure and increased lung accumulation were achieved.

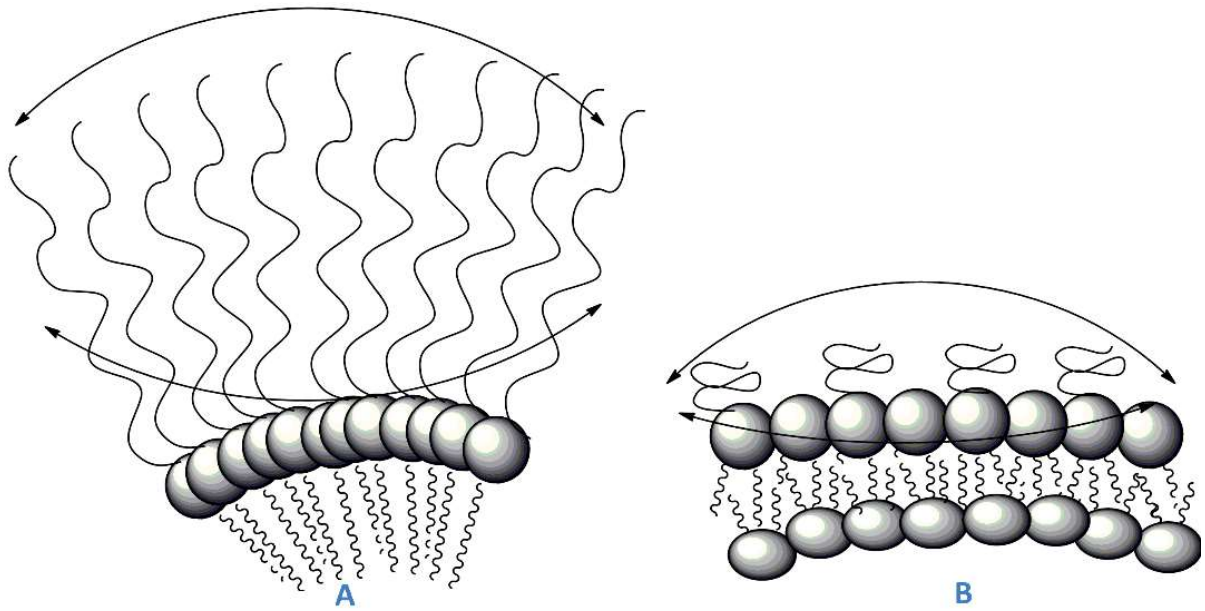

Figure 2. When the surface of the nanoparticle is coated with PEG, two situations may happen: (A), highly bent particle's surface and high PEGylation give these chains a "brush" look which ensures thickness of the stagnant water layer that covers the surface and (B), less bent particle's surface and insufficient PEGylation give these chains a "fungus" appearance, and causes the surface water layer to be thin and discontinuous, which is insufficient to protect the particle against opsonization.

Passive targeting of nanodrugs after intravenous administration

As free drug molecules enter the bloodstream through injection or oral route and since they are very small in size, they pass the spaces between the endothelial cells (3-4 nm) of the blood vessels and distribute throughout the body. However, since nanodrugs are larger in size than the spaces between blood vessels, they remain in the circulation for a longer time, especially when their surface is coated with PEG. As a result, the probability of drugs in nano-carriers reaching the diseased area is much higher than free drugs.

In cancer and inflammatory tissues, blood vessels are highly permeable and the gaps among them are larger than 100 nm and sometimes even 800 nm in size. Nanoparticles that reach the affected region pass to the diseased tissue through the gaps in these vessels and stay there (Enhanced Permeability and Retention (EPR)). Drug molecules are then released from the carriers and exert the desired pharmacological effects (Fig. 3). This mechanism is referred to as delivering the drug to the target by passive mechanism (28). This method is one of the most successful instances of application of nano-technology in medicine.

Surface of nanoparticles may also be functionalized with different targeting moieties that interact with the specific markers in the region (92) .This active targeting results in increased drug accumulation in the target cells. Active targeting mechanism and other targeting methods are outside the scope of this review, but the reader may refer to other publications for more information (93). The targeted nanoparticles may not only show a higher effect due to increased drug concentration and specificity at the diseased tissues, but also reduce or even eliminate toxicity and side effects of the drug. This is due to the facts that drug in nanocarriers cannot extravasate the healthy vessels to permeate into healthy tissues, and also does not interact with blood cells in the circulation.

CONTRIBUTION OF NANOMEDICINES TO SOCIAL WELFARE, HEALTH AND ECONOMY

The contributions of nanotechnology to the medical field are important for the society from many perspectives. This technology provides new products, from visualization to diagnosis and every area of treatment, and contributes to the health care and economics of the society in many ways as summarized in Table 2.

Table 3 and 4 are representing the majority of examples of nanoformulations in cancer therapy. The active ingredients, company/sponsor, indications, status, application and trial numbers linked to source of information in FDA, EMA or other related recourses for further information are given.

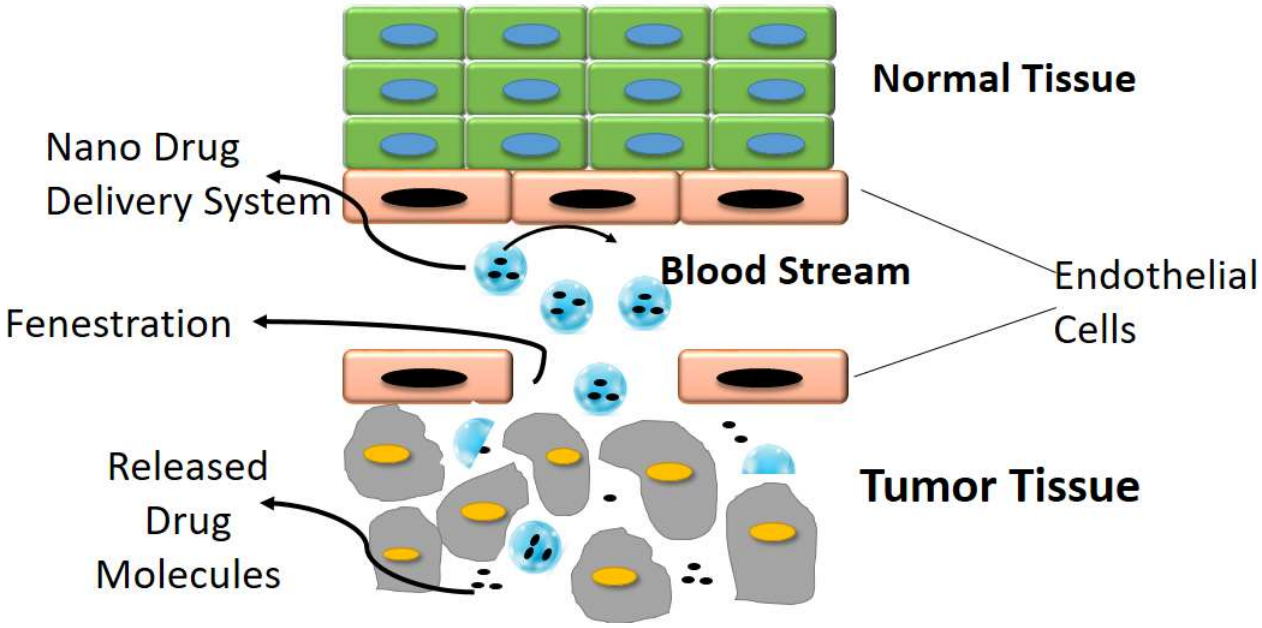

Figure 3. Enhanced Permeability and Retention (EPR) effect.

Table 2. Contribution of nanotechnology to the health care and economy from different perspectives (94)

1. Industrial Perspective

1. Upgrading the added value of high cost biopharmaceuticals
   - Similar effect with lower dose, improved efficacy with the same dose, controlled drug release and improved pharmacokinetic profile can be achieved.
2. Reformulation of existing drugs
   - Increase of drug half-life, reusing the drugs that have serious side effects and repositioning may be possible

2. Clinical Perspective

1. Possibility of more effective and less toxic interventions
2. Patient-friendly approaches
3. Personalized treatments
4. Possibility of using targeted medicines
5. Accelerating the treatment process
6. Achieving better results in patients' complaints and physical appearance
7. Improvement of medical and pharmaceutical care

3. Health Care System Perspective

1. Allowing rational drug use
2. Reduction of health expenditures in general
   - Increasing the drug efficacy, prolonging the half-life, reduction of personal health care costs and the effective treatment of common, expensive diseases
3. Improving the quality of health care services

4. Patient Perspective

1. Decreasing dose frequency and prolongation of dose intervals
2. Ability to be applied by minimally invasive methods
3. Receiving maximum result from treatment
4. Reducing side effects
5. Increasing life quality of the patient

Table 3. Examples of approved and in clinical trial Liposomal formulations.

Product name	Active ingredient	Company/ Sponsor	Indication	Status	Source
DaunoXome	Daunorubicin	Galen	AIDS-related Kaposi's sarcoma, metastatic ovarian cancer, metastatic breast cancer, multiple myeloma	FDA Approved 1996	New Drug Application (NDA): 050704
Doxil/Caelyx	Doxorubicin Hcl	Janssen	AIDS-related Kaposi's sarcoma, Acute myeloid leukemia, ovarian cancer	FDA Approved 1995	New Drug Application (NDA): 050718
DepoCyte	Cytarabine	Depotech Corporation Sigma-Tau	Lymphomas or leukemia with meningeal spread add Neoplastic meningitis	FDA accelerated approval in 1999 and full approval in 2007	Application No.: 21-041
Onivyde (MM-398)	Irinotecan	Merrimack Pharmaceutical, Inc.	Pancreatic cancer	FDA Approved 2015	Application No.: 207793
Marqibo (vinCRIStine sulfate LIPOSOME injection)	Vincristine	Talon Therapeutics, Inc.	Acute lymphoid leukemia	FDA Approved 2012	Application No.: 202497
Vyxeos™/daunorubicin	Daunorubicin and Cytarabine	Jazz Pharmaceuticals plc	Acute Myeloid Leukemia	FDA Approved 2017	Application No.: 209401

Table 3. Continued…

MEPACT	Mifamurtide acting	IDM Pharma	Osteosarcoma	EMA Approved 2013	EMEA/H/C/000802
Myocet	Doxorubicin liposome	Teva UK	Breast	EMA Approved (2000)	EMEA/H/C/000297
CPX-1	Irinotecan	Jazz Pharmaceuticals	Colorectal cancer or colon cancer	Phase II	NCT00361842
Liposome Encapsulated SN38 (LE-SN38)	Sn-38	Insys therapeutics inc ph1 (cli trials)	Neoplasms	Phase II	NCT00046540
Lipoplatin	Cisplatin	Centre Hospitalier Universitaire Vaudois	Non small cell lung cancer (NSCLC), breast cancer, gastric cancer	Phase III	NCT02702700
Cisplatin liposomal (SLIT Cisplatin)	Cisplatin	Insmed Incorporated	Osteosarcoma Metastatic	Phase I Phase II	NCT00102531
Liposomal-Cisplatin Analogue (L-NDDP)	Cisplatin	NYU Langone Health	Malignant Mesothelioma	Phase II	NCT00004033
Aroplatin	NDDP (bis-neodecanoato- trans-R,R-1,2-diaminocyclohexane plati-num(II))	Aronex Pharmaceuticals	Refractory colorectal can- cer, malignant pleural mesothelioma	Phase II	NCT00081549
liposomal oxaliplatin (MBP-426)	Mbp-426	Mebiopharm co., ltd	Advanced gastro-intestinal cancer	Phase I	NCT00355888
Liposomal Oxaliplatin/Folinic Acid/5-Fluorouracil	Mbp-426/leucovorin/5-fu	Mebiopharm co., ltd	Gastric adenocarcinomagastroesophageal junction adenocarcinomaesophageal adenocarcinoma	Phase II	NCT00964080
Anti-EGFR ILs-DOX	C225-ils-dox	University Hospital, Basel, Switzerland	Glioblastoma	Phase I	NCT03603379
2B3-101	Dox	Bbb-therapeutics b.v.	Glioma	Phase I, II	NCT01386580

Table 3. Continued…

MM-302	Dox	Merrimack pharmaceuticals	Advanced breast cancer	Phase I	NCT01304797
ThermoDox Liposomal Doxorubicin	Dox	Celsion company	Primary hepatocellular carcinoma refractory chest wall breast cancer colorectal liver metastases	Phase III	NCT00617981 NCT02112656 *
Liposomal annamycin	Annamycin	NYU Langone Health	DOX-resistant breast cancer	Phase I, Phase II	NCT00012129
LEM	Mitoxantrone	INSYS Therapeutics Inc	Tumors	Phase I	NCT00024492
SPI-77	Cisplatin	NYU Langone Health /National Cancer Institute (NCI)	Ovarian cancer	Phase II	NCT00004083
LiPlaCis	Cisplatin	Oncology Venture	Phase 1: Advanced or Refractory Solid tumoursphase 2 Part: Metastatic Breast Cancer, Prostate Cancer and Skin Cancer	Phase I, Phase II	NCT01861496
Nanoliposomal CPT-11	Irinotecan	University of California, San Francisco	Glioblastomagliosarcomaanaplastic astrocytomaanaplastic Oligodendroglioma	Phase I	NCT00734682
L9NC	9-nitro-20 (S)-camptothecin	University of New Mexico	Corpus Uteri Lung Cancer	Not applicable	NCT00277082
IHL-305	Irinotecan phase	Yakult honsha co., ltd	Advanced solid tumor	Phase I	NCT00364143
PEP02	Irinotecan	Pharmaengine	Stomach neoplasmsesophageal Neoplasms	Phase II	NCT00813072
TLI	Topotecan	Spectrum Pharmaceuticals, Inc	Solid tumor, ovarian cancer, small cell lung cancer	Phase I	NCT00765973
PNU-93914	Ptx	Memorial sloan kettering cancer center	Esophageal cancer	Phase II	NCT00016900

Table 3. Continued…

LEP-ETU	Ptx	Insys therapeutics inc	Neoplasms	Phase I	NCT00100139
VLI (vinorelbine liposomes)	Vinorelbine	Spectrum Pharmaceuticals, Inc	Tumorshodgkins diseasenon-Hodgkins Lymphoma	Phase I	NCT00364676
CPX-351	Combination of cytarabine and daunorubicin	Jazz Pharmaceuticals	Acute myeloid leukemia	Phase I	NCT04038437
SGT-53	P53 gene	Synergene Therapeutics, Inc.	Solid tumor	Phase I	NCT00470613
LErafAON-ETU	Antisense oligonucleotide	Insys therapeutics inc	Neoplasm	Phase I	NCT00100672
NX 211	Lurtotecan	Astellas Pharma Inc /OSI Pharmaceuticals	Ovarian cancer	Phase II	NCT00046800
LE-DT	Docetaxel	INSYS Therapeutics Inc	Pancreatic cancer,	Phase II	NCT01186731
L-BLP25	Tecemotide	Merck kgaa, Darmstadt, Germany	Carcinoma, Non-Small-Cell Lung Neoplasms	Phase II	NCT00157196
Lipovaxin-MM	3-nitrlotriacetic acid linked to ditetradecylamine lipid liposome with histidine tagged targeting ligands	Lipotek pty ltd/ Royal adelaide hospital/ Trident clinical research pty ltd	Melanoma	Phase I	NCT01052142
BP1001 L-Grb-2 Antisense Oligonucleotide	Grb-2 (Growth factor receptor-bound protein 2)	Bio-Path Holdings, Inc.	Recurrent Adult Acute Myeloid Leukemia Acute Lymphoblastic Leukemia Myelodysplastic Syndrome Ph1 Positive CML	Phase I	NCT01159028

Table 3. Continued…

SPI-077 (Liposomal Cisplatin)	Cisplatin	NYU Langone Health/ National Cancer Institute (NCI)	Ovarian Cancer	Phase II	NCT00004083
OSI-7904L	Thymidylate synthase in-hibitor	OSI Pharmaceuti cals	Locally Recurrent or Metastatic Cancer of the Head and Neck (Must Have Failed First-Line Therapy)	Phase II	NCT00116909
OSI-211	Lurtotecan	Astellas Pharma Inc/ OSI Pharmaceuti cals	SCLC Carcinoma, Small Cell	Phase II	NCT00046787
Rexin-G	Cyclin G1 gene	Epeius Biotechnolo gies	All solid tumors, osteosarcoma and soft tissue sarcoma, Breast cancer	Approved in Philippines 2007/ Phase I, Phase II	NCT00505271*
LEP—ETU	Paclitaxel	Insys	Breast cancer	Phase II	NCT01190982
EndoTAG-1	Paclitaxel	Jules Bordet Institute	Breast cancer	Phase II	NCT01537536
Atragen (tretinoin liposome)	Tretinoin	Weill Medical College of Cornell University	Kidney Cancer	Phase II	NCT00003656
NKTR -102	Irinotecan, pegylated liposome	Nektar Therapeutics	Metastatic Solid Tumors/ Breast /colorectal/ ovarian cancer	Phase III	NCT01492101 NCT02915744 *
* other studies of same formulation in other phases or different cancer types are present					

Table 4. Examples of approved and in clinical trial nanoformulations.

Nanomedicine type	Product name	Active ingredient	Company/Sponsor	Indication	Status	Source
Nanoemulsions	**Oncaspar®**	Pegaspargase (mpeg-asparaginase)	Sigma-tau Arzneimit- tel gmbh Germany	Acute lymphocytic leukemia	EMA Approved 2016	EMEA/H/C/003789
	Zevalin®	90Y-ibritumomab tiuxetan	Bayer Pharma	Lymphoma	FDA Approved 2002	Application No.: 125019
	Zevalin®	Ibritumomab tiuxetan	Spectrum Pharmaceuticals B.V.	Lymphoma, Follicular	EMA Approved 2004	EMEA/H/C/000547

Table 4. Continued…

Metallic NPs	**Aurimmune (CYT-6091)**	TNF-α bound to colloidal Gold nanoparticles	National Institutes of Health Clinical Center (CC)	Adrenocortical Carcinoma, Breast, Colorectal, Gastrointestinal, Kidney, Liver, Ovarian and Pancreatic Cancers Sarcoma and Melanoma	Early Phase I	NCT00436410
	Aurimmune (CYT-6091)	TNF-Bound Colloidal Gold	National Institutes of Health Clinical Center (CC)	Unspecified Adult Solid Tumor	Phase I	NCT00356980
Polymeric NPs	**Adagen**	Pegademase bovine	Leadiant Biosciences	SCID	FDA Approved	New Drug Application (NDA): 019818
	Eligard	Leuprolide acetate and polymer	Atrix Laboratories	Prostate cancer	FDA Approved 2004	Application No.: 021731
	Eligard	Leuprolide acetate	Tolmar	Advanced prostate cancer	Fda approved 2016	NDA 21343/ S33
	Oncaspar	Pegaspargase	Baxalta U.S.	ALL	FDA approved 1994	BLA 103411/S-5196
	Oncaspar	Pegaspargase	Les Laboratoires Servier	Precursor Cell Lymphoblastic Leukemia-Lymphoma	Approved EMA 2016	EMEA/H/C/003789
Polymeric conjugates	**Zinostatin stimalamer Conjugate**	Zinostatin	Yamanouchi	Primary unresectable hepatocellular carcinoma	Approved in Japan 1993	Resource
	CRLX101 (cyclodextrin adamantane)	CRLX101Drug	Cerulean Pharma Inc.	Rectal Cancer	Phase II	NCT02010567

Table 4. Continued…

	Crlx101 (cer ulean)	Crlx101 (cerulean) bevacizumab	Abramson cancer center of the university of pennsylvania	Renal cell carcinoma	Phase I	NCT01625936
	CRLX101, a cyclodextrin Olaparib	CRLX101 Olaparib	National Cancer Institute (NCI)	Urothelial cancer, NSCLC SCLC, prostate Cancer	Phase I Phase II	NCT02769962
	XMT1001 (fleximertm)	Camptothecin	Mersana Therapeutics	Small Cell Lung cancernon-small Cell Lung Cancer	Phase I	NCT00455052
	Cpc634	Cripec® nanopart icles with docetaxel (taxotere®)	Cristal therapeutics	Ovarian cancer	Phase II	NCT03742713
Polymeric micelles	**Genexol-PMTM**	Paclitaxel	Samyang Biopharmaceu ticals	Breast cancer; Non-small cell lung cancer	Approved in south Korea 2006	Resource
	Genexol-PMTM	Paclitaxel	Samyang Biopharmaceu ticals	Gynecologi c Cancer	Phase I	NCT02739529
	Nk105	Paclitaxel	Nippon kayaku co., ltd.	Breast cancer nos metastatic recurrent	Phase III	NCT01644890
	Nc-4016	Nc-4016	Nanocarrier co., ltd.	Advanced cancerlymp homa	Phase I	NCT03168035
	Nanoxeltm	Paclitaxel	Samyang Biopharmaceu ticals	Advanced breast cancer	Approved in 2013 South Korea	Resource1 Resource 2
	Nanoplatin (NC-6004) and Gemcitabin e	Nanoplatin and Gemcitabine	Nanocarrier C o., Ltd.	Locally Advanced and Metastatic Pancreatic Cancer	Phase I Phase II	NCT00910741

Table 4. Continued…

	PROMITIL (Pegylated liposomal mitomycin-C)	Promitil Capecitabinebevacizumab	Lipomedix Pharmaceuticals Inc.	Cancersolid tumormetastatic Colorectal Cancer (mcrc)	Phase I	NCT01705002
	Oncoprex	FUS1 (TUSC2) encapsulated Liposome	Genprex, Inc.	Lung cancer	Phase I Phase II	NCT01455389
	E7389-e044-112 (eribulin-liposomal formulation)	Eribulin-lf	Eisai limited	Solid tumors	Phase I	NCT01945710
	188Re-BMEDA-liposome	Radiation	Institute of Nuclear Energy Research, Taiwan	Tumors	Phase I	NCT02271516
	JVRS-100 (Cationic liposome Plasmid DNA complex)	JVRS-100	Milton S. Hershey Medical Center	Leukemia	Phase I	NCT00860522
	Lipocurc	Liposomal curcumin	Signpath Pharma	Patients with Advanced Cancer Who Have Failed Standard of Care Therapy	Phase I/II	NCT02138955
	Lipusu®	Paclitaxel liposome	Nanjing luye sike pharmaceutical co., ltd.)	Breast cancer	Phase IV	NCT02142790
	Lipusu®	Paclitaxel liposome Gemcitabine Cisplatin	Nanjing luye sike pharmaceutical co., ltd.	Lung squamous cell carcinoma	Phase IV	NCT01994031
	Tkm-080301	Arbutus biopharma	Lipid particle targeting polo-like kinase1 (plk1) for delivery of sirna	Colorectal, Pancreas, Gastric, Breast and Ovarian cancers with hepatic metastase	Phase I	NCT01437007 *

Table 4. Continued…

	Cynviloq IG-001 (Paclitaxel polymeric micelle nanoparticl)	Nab-paclitaxel IG-001	Sorrento Therapeutics, Inc.	Metastatic Breast cancerlocally Recurrent Breast Cancer	Not Applicable	NCT02064829
	Nanoxel M (Docetaxel-PM)	Docetaxel micelle	Samyang Biopharmaceuticals Corporation	Head and Neck Squamous Cell Carcinoma	Phase II	NCT02639858
Crystalline NPs	**Nbtxr3 A suspension of nanoparticles composed of hafnium oxide crystallites and phosphate groups in an aqueous medium**	Device: nbtxr3	Nanobiotix	Head and neck cancer	Phase I	NCT01946867
	Targomirs	Targeted minicells containing a microrna mimic	Asbestos Diseases Research Foundation/ Engeneic Limited	Malignant Pleural Mesothelioa Non-Small Cell Lung Cancer	Phase I	NCT02369198
	Sgt-94	Rb94 plasmid DNA in a liposome with anti-transferrin receptor antibody	Synergene therapeutics	Neoplasm	Phase I	NCT01517464
	Magnablate Iron NPs	Magnetic Nanoparticle Injection	University College London Hospitals	Prostate Cancer	Early Phase I	NCT02033447
Protein NPs	**Abraxane**	Albumin-bound paclitaxel	American Pharmaceutical Partners, Inc. / American Bioscience, Inc.	Breast Cancer, Non-Small Cell Lung Cancer, Pancreatic Cancer	FDA Approved 2005	Application No.: 021660

Table 4. Continued…

	Abraxane	Nab paclitaxel in combination with gemcitabine	Celgene	Metastatic pancreatic cancer	EMA Approved 2013	EMEA/H/C/000778
	Ontak	Denileukin diftito	EISAI INC	Cutaneous T-cell lymphoma	FDA Approved 1999	Biologic License Application (BLA): 103767
	Ontak	DENILEUKIN DIFTITOX	EISAI INC	Cutaneous T-cell lymphoma	EMA Approved 2002	EU/3/01/075
	Kadcyla® KADCYLA ™ (ado-trastuzumab emtansine)	The humanized monoclonal antibody trastuzumab covalently linked to the cytotoxic agent DM1	Genentech, Inc	Metastatic breast cancer	FDA Approved 2013	BLA 125427/0
Virosomes	**Gendicine®**	Wildtype-p53 (rad-p53)	Saudi Food and Drug Authority	Tumors which have mutated p53 genes	2003 Approved by Chinese State Food and Drug Administration	Resource
Micelles	**Nk105**	Paclitaxel	Nanocarriertm	Advanced stomach cancer; breast cancer	Phase III	NCT01644890
	Nc6004	Cisplatin	Nanocarrier co., ltd.	Head and neck cancer	Phase II	NCT03771820
	Paclical	Paclitaxel micelles	Oasmia Pharmaceutical AB	Ovarian cancer	Phase III	NCT00989131
Dendrimer	Dendrimer conjugated AZD4320	AZD0466	AstraZeneca	Advanced Solid Tumors Lymphoma Multiple Myeloma Hematologic Malignancies	Phase I	NCT04214093
	docetaxel (DTX)-dendrimer conjugate	DTXSPL8783	-	advanced solid tumours	Phase I, II	IRAS ID: 204296

* other studies of same formulation in other phases or different cancer types are present

During clinical trials, the distribution and effect of drug-induced molecules on the body are examined in two main areas:

1. *Pharmacokinetics*: The fate of the drug in the body (absorption, distribution, metabolism and excretion) is examined. In other words, the answer to the question "what the body does to the drug?" is given.
2. *Pharmacodynamics* (also known as toxicodynamics): It examines the pharmacological effect of the drug as well as its side effects. In other words, "what the drug does to the body" is examined.

Altered Pharmacokinetics to Improve Properties

Drug administered systemically (such as oral or intravenous) is transported to the organ and tissue through the blood circulation via pharmacokinetic processes. The drug molecules that go out from capillary to the tissues show activity when they bind to their targets or receptors in diseased cells. The efficacy of the drug depends on the amount of the drug molecules present in the target tissue.

With classical drug formulations, the active ingredient is carried in the blood either as a free molecule or it is bound to albumin. Due to this, higher distribution of the drug in the tissues is obtained with conventional drug formulations compared to nanomedicines.

The same active ingredient given at the same dose in a nanocrrier as a nanomedicine can accumulate in the diseased tissue more intensely than the conventional drug formulation due to EPR effect, and may have a much higher effect. This reduces the concentration and possible side effects of the drug on healthy tissues.

Nanomedicines are usually covered with PEG and can stay longer than conventional drugs in the blood circulation. They are also less distributed throughout the body, which extends the half-life of the drug. In addition, since nanodrugs are larger in size than the endothelial cell spaces (<10 nm) in the kidneys, they also differ in their excretion process. Liver metabolism of nanomedicines coated with PEG can also be different from free drugs (Table 5).

In summary, the pharmacokinetic properties (half-life, distribution, elimination, and metabolism) of an active agent in the form of a nanomedicine vary greatly from that of the active ingredient in the form of a free molecule. The use of conventional methods and formulas for the calculation of the PK parameters of nanomedicines may not give accurate results, hence new methods and formulas for nanoparticles need to be developed.

Altered Pharmacodynamics

Pharmacodynamics is the science that studies the biochemical and physiological effects of drugs on the body and their mechanisms of action. The vast majority of drugs show their effect by interacting with different structures (targets or biomarkers) in the diseased organism. The drug target is usually macromolecule, such as a receptor, found on cell membranes and the vast majority of the target macromolecules are protein structures.

The extend of the drug effect depends on the amount of the drug present in that tissue. As the dose or concentration of the drug increases, the effect it creates also increases, but up to a certain point. Increasing the dose or concentration of the drug after this level, no additional biological response can be obtained, due to receptor saturation (95).

In order to be able to exert the action, the active agent of nanomedicine must first be released in a free and active form in the target site. This process depends on the ability of the nano-carrier to carry the drug molecules without releasing them in blood and release all the drug, either in a controlled way or abruptly, in the target tissue.

As a result, the properties of the nano-carrier can indirectly cause changes in the pharmacological effects of the drug. However, the therapeutic index of the active ingredient given to the body as a well-designed nanomedicine will be much larger because of decrease in toxic effects and high accumulation of the active substance in the target tissue. Table 5 attempts to summarize all of these points.

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Table 5. Pharmacodynamic and Pharmacokinetic Events from Conventional Small Drug Molecule Formulations and Drug Molecules Loaded into a Nano Drug Delivery System (96-100)

	Free Small Molecule Formulation	Molecules Loaded into Nano Drug Delivery System (Nanomedicines)
Pharmacodynamic	Dispersed throughout the body from application site and are likely to interact with the cells they are exposed to. Their solubility in water and membrane permeability determine how they reach the target tissue.	Drug is transported in the carrier for a long time without release. A major part of the release occurs in the target organ. While transported in circulation, the free drug molecule is found much less than the loaded dose, reducing the drug and blood cell interaction.
	Drug transported from capillary vasculars to all tissues, and shows its effect by binding to the receptors in the membrane or inside the target cells. Off target side-effect is possible.	Upon removal from the vasculature, the nanomedicine accumulates only in the target tissue (such as tumor or inflamed tissue).[42] Side effects are minimized.
	Activity of the drug depends on the concentration present in that tissue. Drug amount reaching the target tissue is small due to wide biodistribution plus the metabolic and enzymatic reactions.	Drug molecules in the nano drug delivery systems are protected from most metabolic and enzymatic reactions, reaching their target and acting much more effectively.[43]
	Dosage that shows effect (average effective dose, ED50) in 50% of the population is determined by considering the change in genotype enzyme species across the population.	The average effective dose may be lower because the drug molecules in the carrier can not interact the genotype enzymes outside of the target cells.[43]
Pharmacokinetics	Absorption: Absorption refers to the passage of drug from the site of administration to blood or lymph circulation. Based on drug properties, it can have two mechanisms: passive diffusion and active transport.	Drug absorption and protection in GI depends on the properties of the carrier, not the drug.[44]
	Distribution: Drug distribution is dependent on solubility and generally transported by binding to large proteins such as albumin.	Nanocarrier provides favorable protective environment for the drug molecule and prevents its interaction with neighboring proteins and other particles in blood. [45]
	Metabolism: Metabolism is chemical conversion of drug to other substances by the liver or other tissues. Free drugs may undergo significant first pass metabolism in the liver leading to decreased activity.	Nano drug carrier systems and the drug molecules are metabolized by the enzymatic reactions after entering the cell through endocytosis in the target region.
	Elimination: Metabolized drugs are excreted in urine and faces. Some drugs can stay in tissues for a longer time depending on physicochemical properties.	In general, the drug and carrier are metabolized in the target tissue and are excreted through natural ways. Opsonization is another way that causes the nanodrug to be thrown away without reaching the target. Coating the nanoparticles with PEG greatly reduces this possibility. In addition, the size of the nanoparticle must be large enough not to be eliminated by Glomerular Filtration.[46]

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